

Three new species of *Candolleomyces* (Agaricomycetes, Agaricales, Psathyrellaceae) from the Yanshan Mountains in China

Hao Zhou¹, GuiQiang Cheng¹, XiMei Sun¹, RuiYi Cheng²,
HongLiang Zhang², YanMin Dong², ChengLin Hou¹

1 College of Life Science, Capital Normal University, Beijing, 100048, China **2** Beijing Songshan National Nature Reserve Management Office, Beijing 102115, China

Corresponding author: ChengLin Hou (chenglin-hou@cnu.edu.cn)

Academic editor: María P. Martín | Received 30 January 2022 | Accepted 30 March 2022 | Published 13 April 2022

Citation: Zhou H, Cheng GQ, Sun XM, Cheng RY, Zhang HL, Dong YM, Hou CL (2022) Three new species of *Candolleomyces* (Agaricomycetes, Agaricales, Psathyrellaceae) from the Yanshan Mountains in China. MycoKeys 88: 109–121. <https://doi.org/10.3897/mycokeys.88.81437>

Abstract

Three new species, *Candolleomyces incanus*, *C. subcandolleanus* and *C. yanshanensis*, were found and described from Yanshan Mountains in China. The identification is based on morphological observation combined with phylogenetic analysis of ITS-LSU-*Tef1a*-*TUB2*. This study enriched the species diversity of *Candolleomyces* in Yanshan Mountains and provided important data support for the systematic study of *Candolleomyces* in the future.

Keywords

molecular systematics, new taxon, *Psathyrellaceae*, taxonomy

Introduction

Candolleomyces Wächter & A. Melzer was established in 2020, belonging to Basidiomycota, Agaricomycetes, Agaricales, Psathyrellaceae (Wächter and Melzer 2020). In a previous study, this genus was subordinate to *Psathyrella* (Fr.) Quél. (1872) and molecular sequence data have improved understanding of relationships of *Psathyrella* species (Hopple and Vilgalys 1999; Moncalvo et al. 2002; Matheny et al. 2006). However, the combination analysis of the ITS and LSU regions showed that the delimitation of some species within *Psathyrella* are still unclear (Larsson and Örstadius 2008). In more

recent studies, multi-gene loci (for example, ITS, LSU, *Tef1a* and *TUB2*) became the main methods for identification of *Psathyrella* (Wang and Bau 2014; Yan and Bau 2017, 2018a, 2018b, 2021; Yan 2018; Yan et al. 2019).

In previous studies of *Psathyrella*, there are approximately 100 taxa lacking pleurocystidia, but this feature has not been used as a key distinguishing feature (Fries 1838; Smith 1972; Kits van Waveren 1985; Örstadius and Kundsén 2012; Battistin et al. 2014). Based on extensive specimen collection, morphological studies and phylogenetic analyses, *Candolleomyces* has been separated from *Psathyrella* as a new genus and it differs from *Psathyrella* s.s. in lacking pleurocystidia. (Wächter and Melzer 2020).

Currently, there are 25 recognised species in *Candolleomyces* in the Index Fungorum website (<http://www.indexfungorum.org>, until Jan. 2022) and 10 species were reported in China (Yan 2018; Bau and Yan 2021).

Yanshan Mountains are located in North China and have a warm temperate continental monsoon climate, with higher plant diversity. The dominant plants include *Quercus* spp., *Betula* spp., *Abies* spp. and *Pinus tabulaeformis* Carr. et al. (Wang et al. 2021). There is no information about *Candolleomyces* as yet. In this study, based on morphological characters and the phylogenetic analyses, three new species of *Candolleomyces* from Yanshan Mountains in China are described.

Materials and methods

Morphological studies

Collections were obtained and photographed in the field from Yanshan Mountains in China from 2017 to 2020. The collected specimens were dehydrated with a dryer (Dorrex) at 50 °C and the specimens were deposited in the Herbarium of the College of Life Science, Capital Normal University, Beijing, China (BJTC). Macroscopic characters were recorded from specimens. Microscopic characters were observed in thin sections of specimens mounted in 3% potassium hydroxide (KOH) or sterilised water. The shape and size of microscopic structures were observed and noted using a light microscope [Olympus DP71, Tokyo, Japan]. The measurements and Q values are given as (a)b–c(d), in which “a” is the lowest value, “b–c” covers a minimum of 90% of the values and “d” is the highest value. Q stands for the ratio of length and width of a spore (Bau and Yan 2021). Nomenclatural details were submitted to the MycoBank. In this study, the morphological colour comparison was compared to the reference website colorhexa (<https://www.colorhexa.com>).

DNA extraction PCR amplification and sequencing

DNA extraction was achieved by the M5 Plant Genomic DNA Kit [Mei5 Biotechnology, Co., Ltd, China]. The purified DNA was dissolved in 1 × TE buffer and stored at – 20 °C for later use. The PCR amplifications were performed in Bio-Rad S1000 TM

Thermal Cycler [Bio-Rad Laboratories, Inc, USA]. The primer sets ITS1/ITS4 (White et al. 1990) were used to amplify the rDNA ITS region, LR5/LR0R (Vilgalys and Hester 1990) were used to amplify the large subunit nuclear ribosomal DNA (nuLSU rDNA) region and EF983F/EF2218R (Örstadius et al. 2015) were used to amplify the translation elongation factor subunit 1 alpha (*Tef1a*) region. The primer sets B36f and B12r (Nagy et al. 2011) were used to amplify the β -tubulin gene (*TUB2*) region. PCRs were performed in a volume of 25 μ l consisted of 2 μ l of DNA template; 1 μ l of (10 μ M) per primer; 12.5 μ l 2 \times Master Mix [Mei5 Biotechnology, Co., Ltd, China]. PCR amplification conditions refer to Bau and Yan (2021). DNA sequences were sequenced by Zhongkexilin Biotechnology, Co., Ltd, Beijing, China.

Molecular data analyses

The generated raw reads of the DNA sequences were used to obtain consensus sequences using SeqMan v.7.1.0 in the DNASTAR Lasergene Core Suite software (DNASTAR Inc., Madison, WI, USA). All sequences were aligned using MAFFT v.6 (Kato and Toh 2010) and trimmed manually using MEGA 6 (Tamura et al. 2013). For phylogenetic analyses, newly-obtained sequences and additional reference sequences of *Candolleomyces* species were included in the dataset of combined ITS-LSU-*Tef1a*-*TUB2* multi-locus DNA (Table 1), with *Psathyrella multipedata* (Peck) A.H. Sm. (LÖ237-04) used as outgroup. Phylogenetic analyses were performed using PAUP v.4.0b10 for Maximum Parsimony (MP) analysis (Swofford 2003) and MrBayes v.3.1.2 for Bayesian Inference (BI) analysis (Ronquist and Huelsenbeck 2003). ML gene-trees were estimated using the software RAxML 7.4.2 Black Box (Stamatakis 2006; Stamatakis et al. 2008; Zhou and Hou 2019; Zhou et al. 2021).

Maximum Parsimony analysis was performed by a heuristic search option of 1000 random-addition sequences with a tree bisection and reconnection (TBR) algorithm. Maxtrees were set to 1000, branches of zero length were collapsed and all equally parsimonious trees were saved. Other calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC) (Zhou and Hou 2019).

Maximum Likelihood analysis was performed with a GTR site substitution model (Guindon et al. 2010). Branch support was calculated with a bootstrapping (BS) method of 1000 replicates (Hillis and Bull 1993). Bayesian Inference (BI) analysis, using a Markov Chain Monte Carlo (MCMC) algorithm, was performed (Rannala and Yang 1996). MrModeltest v. 2.3 was used to estimate the best model. Two MCMC chains were run from random trees for 10,000,000 generations and stopped when the average standard deviation of split frequencies fell below 0.01. Trees were saved for each 1000 generations. The first 25% of trees were discarded as the burn-in phase of each analysis. Branches with significant Bayesian posterior probabilities (BPP) were estimated in the remaining trees (Posada and Crandall 1998).

The combined alignment and phylogenetic tree were submitted on TreeBASE (www.treebase.org, study 29579).

Table 1. Sequences information used in the phylogenetic analysis in this study.

| Taxa | Voucher | Locality | ITS | LSU | <i>β-Tub</i> | <i>tef-1a</i> |
|------------------------------------|------------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|
| <i>Candolleomyces aberdarensis</i> | GLM-F116094 | Kenya | MH880928 | – | – | – |
| <i>C. albipes</i> | DED8340 | Sao Tome | KX017209 | – | – | – |
| <i>C. badhyzensis</i> | 79478 (TAA) Type | Turkmenistan | KC992883 | KC992883 | – | – |
| <i>C. badiophyllus</i> | SZMC-NL-2347 | – | FN430699 | FM876268 | FN396261 | FM897252 |
| <i>C. cacao</i> | SFSU DED 8339 | Sao Tome | NR148106 | – | – | – |
| <i>C. cacao</i> | FP1R4 | USA | KU847452 | – | – | – |
| <i>C. cacao</i> | MP2R2 | USA | KU847436 | – | – | – |
| <i>C. candolleanus</i> | LAS73030 Neotype | Sweden | KM030175 | KM030175 | – | – |
| <i>C. cladii-marisci</i> | CLUF302 Type | Italy | MK080112 | | | |
| <i>C. efflorescens</i> | Pegler2133 (K) | Sri Lanka | KC992941 | – | – | – |
| <i>C. eurysporus</i> | GLM-F126263 Type | Germany | MT651560 | MT651560 | – | – |
| <i>C. incanus</i> | BJTC Z777 Type | China: Beijing | ON042759 | ON042766 | ON098513 | ON098508 |
| <i>C. incanus</i> | BJTC S173 | China: Beijing | ON042760 | ON042767 | ON098514 | ON098509 |
| <i>C. leucotephrus</i> | LÖ138-01 (UPS) | Sweden | KC992885 | KC992885 | KJ664865 | KJ732775 |
| <i>C. luteopallidus</i> | Sharp20863 (MICH) Type | USA | KC992884 | KC992884 | – | – |
| <i>C. luteopallidus</i> | HMJAU5148 | China: Jilin | MG734736 | MW301084 | MW314056 | MW314073 |
| <i>C. secotioides</i> | UES2918 Type | Mexico | KR003281 | KR003282 | – | KR003283 |
| <i>C. singeri</i> | HMJUA37867 | China: Jilin | MG734718 | MW301088 | MW314059 | MW314077 |
| <i>C. singeri</i> | HMJAU37877 | China: Chongqing | MW301073 | MW301091 | MW314062 | MW314080 |
| <i>C. subcacao</i> | HMJAU37807 Type | China: Henan | MW301064 | MW301092 | MW314063 | MW314081 |
| <i>C. subcacao</i> | HMJAU37808 | China: Henan | MW301065 | MW301093 | MW314064 | MW314082 |
| <i>C. subcacao</i> | HFJAU1014 | China: Jiangxi | MW559218 | – | – | – |
| <i>C. subcacao</i> | HFJAU1274 | China: Jiangxi | MW559219 | – | – | – |
| <i>C. subcacao</i> | HFJAU1488 | China:Anhui | MW559220 | – | – | – |
| <i>C. subcandolleanus</i> | BJTC Z239 Type | China: Tianjin | ON042755 | ON042762 | ON098510 | ON098505 |
| <i>C. subcandolleanus</i> | BJTC Z232 | China: Tianjin | ON042756 | ON042763 | – | – |
| <i>C. subminutisporus</i> | HMJAU37801 Type | China: Hubei | MW301066 | MW301094 | MW314065 | MW314083 |
| <i>C. subminutisporus</i> | HMJAU37916 | China: Henan | MW301067 | MW301095 | MW314066 | MW314084 |
| <i>C. subsingeri</i> | HMJAU37811 Type | China: Jilin | MG734715 | MW301097 | MW314067 | MW314085 |
| <i>C. subsingeri</i> | HMJAU37913 | China: Jilin | MG734725 | MW301098 | MW314068 | MW314086 |
| <i>C. sulcatotuberculosis</i> | GB:LO55-12 | – | KJ138422 | KJ138422 | – | – |
| <i>C. sulcatotuberculosis</i> | HFJAU1515 | China: Fujian | MW375696 | – | MW382967 | MW382965 |
| <i>C. sulcatotuberculosis</i> | Chiarello 07-10-2013 | – | KJ138423 | – | – | – |
| <i>C. trinitatensis</i> | TL9035 (C) | Ecuador | KC992882 | KC992882 | KJ664863 | – |
| <i>C. trinitatensis</i> | ADK4162 (BR) | Togo | KC992886 | KC992886 | – | – |
| <i>C. yanshanensis</i> | BJTC Z783 | China: Beijing | ON042757 | ON042764 | ON098511 | ON098506 |
| <i>C. yanshanensis</i> | BJTC Z110 Type | China: Beijing | ON042758 | ON042765 | ON098512 | ON098507 |
| <i>Candolleomyces</i> sp. | BAB-4773 | India | KP686450 | – | – | – |
| <i>Candolleomyces</i> sp. | BAB-5172 | India | KR349656 | – | – | – |
| <i>Candolleomyces</i> sp. | BAB-4748 | India | KR154977 | – | – | – |
| <i>Candolleomyces</i> sp. | BAB-4747 | India | KR154976 | – | – | – |
| <i>Candolleomyces</i> sp. | BAB-5202 | India | KT188611 | – | – | – |
| <i>Psathyrella multipedata</i> | LÖ237-04 | Sweden | KC992888 | KC992888 | KJ664867 | KJ732777 |

Notes: The new generated sequences are emphasised in bold.

Result

Phylogenetic analyses

For the ITS-LSU- *Tef1a*-*TUB2* sequence dataset, a total of 3459 characters including gaps (694 for ITS, 1316 for LSU, 1023 for *Tef1a*, and 426 for *TUB2*) were included in the

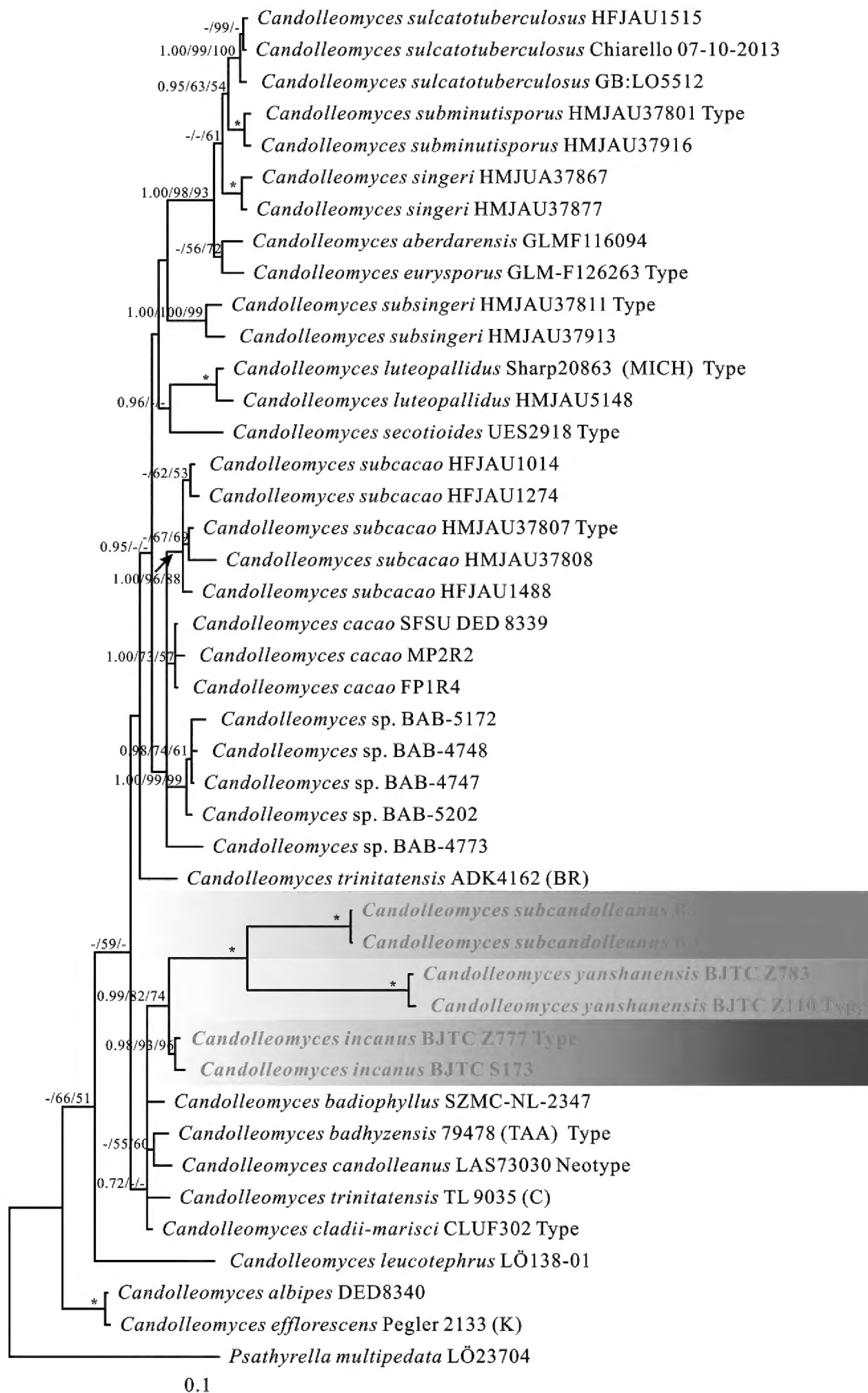


Figure 1. Multi-gene phylogenetic tree obtained from the Bayesian analysis. Numbers above branches are Bayesian posterior probability (pp) values, Maximum Likelihood bootstrap (MLB) and Maximum parsimony bootstrap (MP) values. Asterisks (*) denote branches with pp = 1.00, MLB = 100% and MP = 100%. Numbers above branches represent strongly and moderately support (pp ≥ 0.95, MLB ≥ 50% and MP ≥ 50%). The red font indicates the position of the new species.

phylogenetic analysis. Using RAxML, MrBayes and PAUP to construct ML, Bayesian and MP phylogenetic trees, the results show that the topology and branching order were similar and the Bayesian tree is shown in this paper (Fig. 1). The Maximum likelihood analysed was performed with a GTR model. For the Bayesian analyses, the GTR + I + G models were recommended by MrModeltest. The heuristic search using Maximum Parsimony (MP) generated 1000 parsimonious trees (TL = 1168, CI = 0.768, RI = 0.815, RC = 0.232) and branches of zero length were collapsed and all multiple parsimonious trees were saved.

Based on the results, six specimens were assigned to three branches and were described as three new species. The three new species (*Candolleomyces yanshanensis*, *C. subcandolleanus*, *C. incanus*) and a known species (*Candolleomyces badiophyllus* (Romagn.) D. Wächt. & A. Melzer etc.) clustered together in the phylogenetic tree. The three new species clustered into together (pp = 0.99, MLbs = 82%, MPbs = 74%), but three new species separately formed three subclades with high support value. *Candolleomyces yanshanensis*, *C. subcandolleanus* and *C. incanus* can be distinguished by the phylogenetic tree, sequence base differences and morphological characteristics.

Taxonomy

Candolleomyces yanshanensis C. L. Hou & H. Zhou, sp. nov.

MycoBank No: 843464

Fig. 2

Etymology. *yanshanensis* referred to the locality where the type specimen was collected.

Type. CHINA, Beijing, Changping District, Beitaizi Village, 40.272906°N, 116.4203°E, alt. 149 m, 14 Aug 2019, coll. X.Y. Shen, H Zhou and R.T. Zhang, BJTC Z110.

Diagnosis. *Candolleomyces yanshanensis*, pileus 20–60 mm, flabellate, flattening with age, hygrophanous. Basidiospores $5.8\text{--}8.2 \times 3.3\text{--}5.4 \mu\text{m}$, often with germ pore. Subglobose cell, irregular oval, (18) 20–27 μm broad.

Description. Pileus 20–60 mm, flabellate, flattening with age, hygrophanous, slightly dirty white (#e3dac9) to pale brown (#deb887). Veil white (#ffffff), fibrils in young, evanescent. Context 1.0–2.0 mm broad at centre, same colour as pileus. Lamellae sparsely to moderately, adnate, slightly dirty white (#e3dac9) to champagne (#fad6a5), edge white (#ffffff) as spores mature. Stipes 50–130 \times 3–6 mm, smooth, fibrils on the base, cornsilk (#f0ead6) to white (#ffffff).

Basidiospores $5.8\text{--}8.2 \times 3.3\text{--}5.4 \mu\text{m}$, Q = 1.4–2.0, ellipsoid to long ellipsoid, ovoid to ellipsoid, partly triangular at base, dark brown (#b8860b) to brown (#b06500) in water, smooth, abundant, multi-guttules, often with germ pore. Basidia 17–31 \times 5.8–7.5 μm , short clavate, hyaline, 4-spored. Cheilocystidia 22–35 (40) \times 8–11 (15) μm , irregular utriform or claviform, apex obtuse or broadly obtuse or often subcapitate, rarely with deposits. Pileipellis consists of 2–3 cells deep layer of irregular subglobose cell, irregular oval, (18) 20–27 μm broad.

Habit and habitat. Clumped on the ground with rich humus in broad-leaved forests or broad-leaved shrubs.

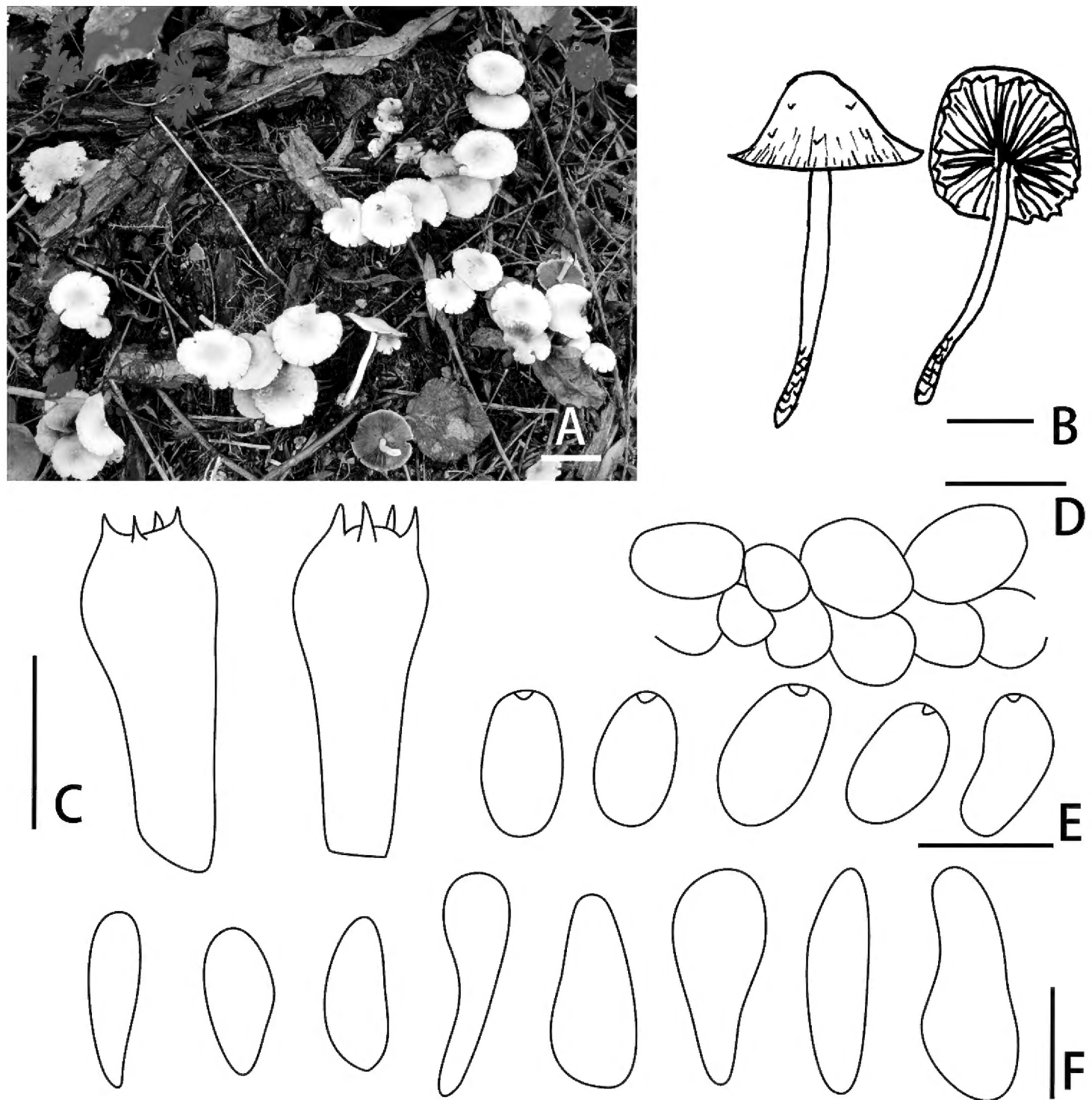


Figure 2. Basidiomata and microscopic features of *Candolleomyces yanshanensis* (BJTC Z110) **A, B** basidiomata **C** basidia **D** pileipellis **E** basidiospores **F** cheilocystidia. Scale bars: 20 mm (**A, B**); 10 μ m (**C**); 20 μ m (**D**); 5 μ m (**E**); 20 μ m (**F**).

Additional specimen examined. CHINA, Beijing, Changping District, Tailing, 40.327397°N, 116.21916°E, alt. 172 m, 17 Aug 2020, coll. X.Y. Shen, H Zhou and X.B. Huang, BJTC Z783.

Candolleomyces subcandolleanus C. L. Hou & H. Zhou, sp. nov.

MycoBank No: 843466

Fig. 3

Etymology. *subcandolleanus* referred to its morphological similarity to *Candolleomyces candolleanus* (Fr.) D. Wächt. & A. Melzer.

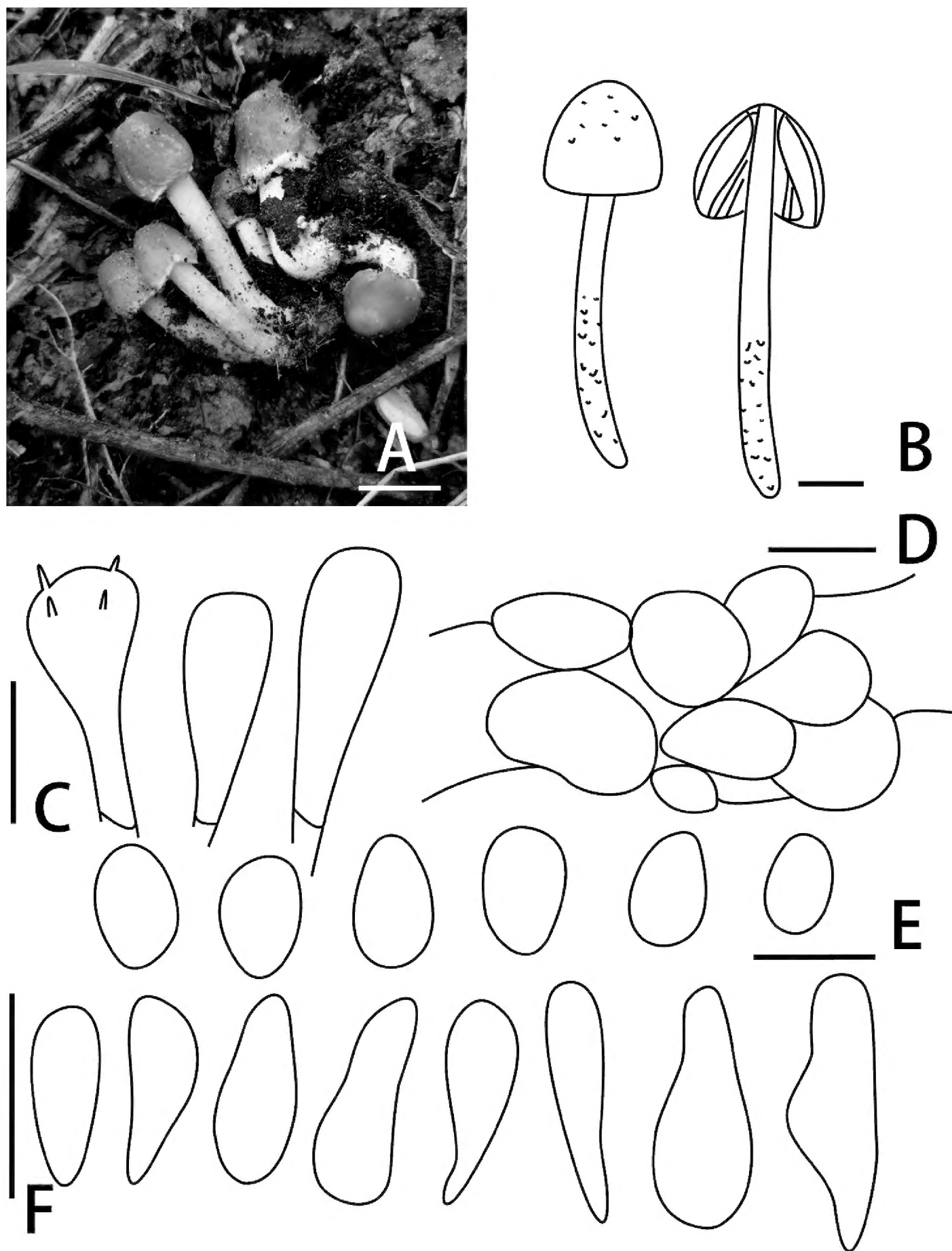


Figure 3. Basidiomata and microscopic features of *Candolleomyces subcandolleanus*. (BJTC Z239) **A, B** basidiomata **C** basidia **D** pileipellis **E** basidiospores **F** cheilocystidia. Scale bars: 10 mm (**A, B**); 10 µm (**C**); 20 µm (**D**); 5 µm (**E**); 20 µm (**F**).

Type. CHINA, Tianjin, Jizhou District, Sanjiebei, 40.227984°N, 117.43354°E, alt. 235 m, 17 Aug 2019, coll. X.Y. Shen, H. Zhou and R.T. Zhang, BJTC Z239.

Diagnosis. *Candolleomyces subcandolleanus*, pileus 5–20 mm. Basidiospores $5.5\text{--}6.7 \times 3.2\text{--}4.5$ µm, germ pore absent. Cheilocystidia $21\text{--}28$ (30) \times $8\text{--}12$ (15) µm. Subglobose cell, irregular oval or long oval, (13) 16–25 µm broad.

Description. Pileus 5–20 mm, campanulate to conical, smooth, fibrils in young, evanescent, brown (#b06500) to golden brown (#996515). Veil white (#ffffff), fibrils in young, evanescent. Context 0.2–0.5 mm broad at centre, same colour as pileus. Lamellae moderately to normally, adnate, slightly dirty white (#e3dac9) to white (#ffffff), edge white (#ffffff) as spores mature. Stipes 20–60 × 1–3 mm, smooth, fibrils on the base, cornsilk (#f0ead6) to white (#ffffff).

Basidiospores 5.5–6.7 × 3.2–4.5 µm, $Q = 1.4–2.0$, ellipsoid to ovoid, pale cream (#fffff0) to pale lemon (#fffacd) in water, smooth, multi-guttules, germ pore absent. Basidia 18–27 × 5–10 µm, short clavate, hyaline, 4-spored. Cheilocystidia 21–28 (30) × 8–12 (15) µm, utriform or claviform, apex obtuse or broadly obtuse or often subcapitate, rarely with deposits. Trama of gills irregular. Pileipellis consists of irregular subglobose cell, irregular oval or long oval, (13) 16–25 µm broad.

Habit and habitat. Clumped on the ground with rich humus in broad-leaved forests or broad-leaved shrubs.

Additional specimen examined. CHINA, Tianjin, Jizhou District, Huangyaguan Great Wall, 40.245615°N, 117.44047°E, alt. 235 m, 17 Aug 2019, coll. X.Y. Shen, H. Zhou and R.T. Zhang, BJTC Z232.

***Candolleomyces incanus* C. L. Hou & H. Zhou, sp. nov.**

MycoBank No: 843465

Fig. 4

Etymology. *incanus* referred to the basidiomata appears incanus.

Type. CHINA, Beijing, Changping District, Sidaohu Village, 40.246374°N, 116.4406°E, alt. 114 m, 16 Aug 2020, coll. X.Y. Shen, H. Zhou and X.B. Huang, BJTC Z777.

Diagnosis. *Candolleomyces incanus*, pileus 5–25 mm, hemispherical to conical. Basidiospores 6.0–7.0 × 3.2–4.5 µm. Stipe 40–70 × 4–6 mm, smooth, germ pore absent. Subglobose cell, irregular oval or long oval, (22) 25–32 µm broad.

Description. Pileus 5–25 mm, hemispherical to conical, hygrophanous, incanus (#f2f3f4) to nude (#fdf5e6). Veil white (#ffffff), fibrils in young, evanescent. Context 0.5–1.0 mm broad at centre, same colour as pileus. Lamellae moderately to normally, adnate, off-white (#f2f3f4) to white (#ffffff), edge white (#ffffff) as spores mature. Stipes 40–70 × 4–6 mm, smooth, hygrophanous, cornsilk (#f0ead6) to white (#ffffff).

Basidiospores 6.0–7.0 × 3.2–4.5 µm, $Q = 1.4–1.9$, ellipsoid, floral white (#fffaf0) to dark yellow (#eedc82) in water, smooth, abundant, multi-guttules, germ pore absent. Basidia 15–20 × 5–8 µm, short clavate, hyaline, 4-spored. Cheilocystidia 17–27 (31) × 7–11 (13) µm, utriform, apex obtuse or broadly obtuse or often subcapitate, rarely with deposits. Trama of gills irregular. Pileipellis consisted of irregular subglobose cell, irregular oval or long oval, (22) 25–32 µm broad.

Habit and habitat. Clumped on the ground with rich humus in deciduous broad-leaved or deciduous coniferous forests.

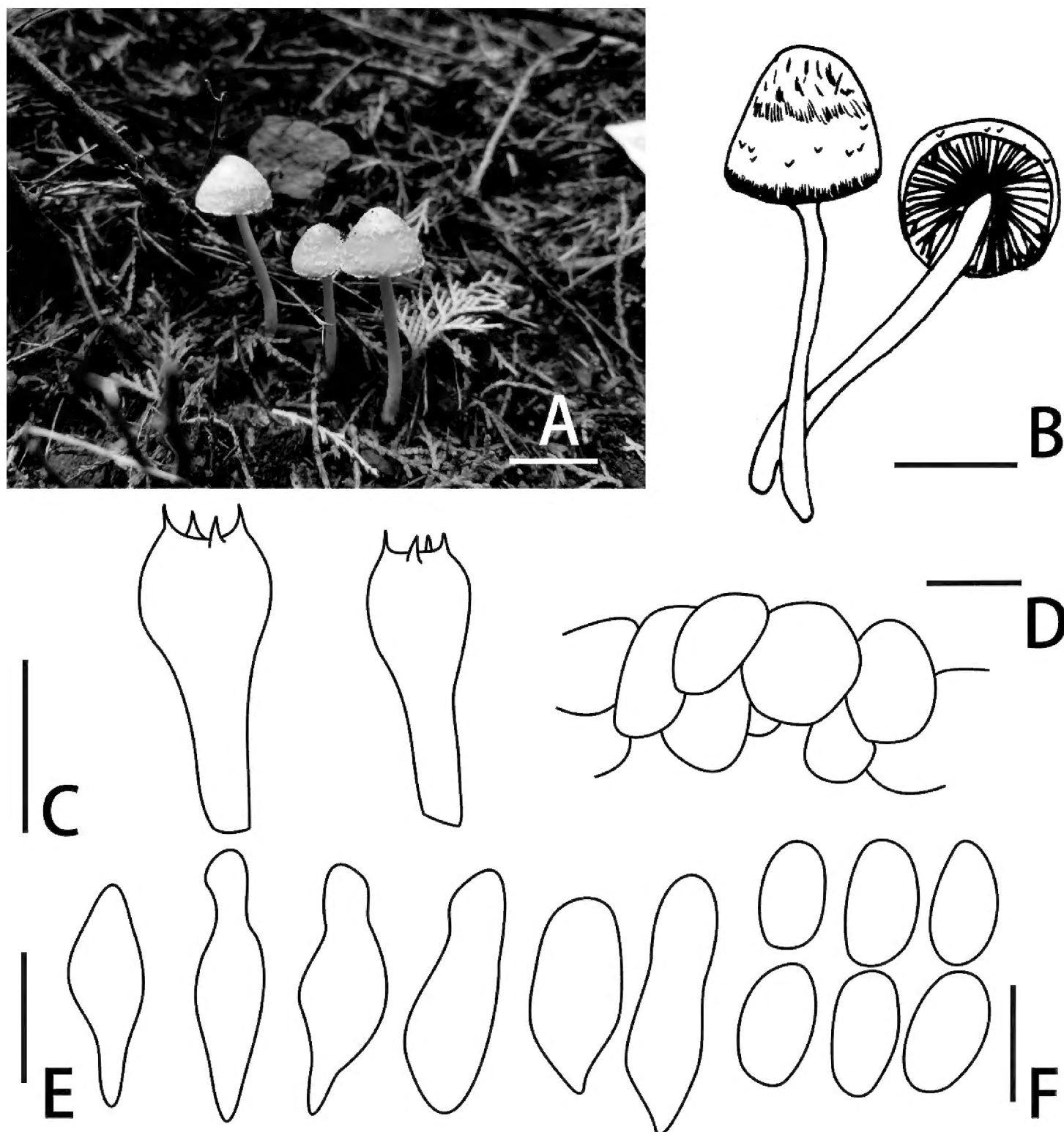


Figure 4. Basidiomata and microscopic features of *Candolleomyces incanus* (BJTC Z777) **A, B** basidiomata **C** basidia **D** pileipellis **E** cheilocystidia **F** basidiospores. Scale bars: 20 mm (**A, B**); 10 μ m (**C**); 20 μ m (**D, E, F**).

Additional specimen examined. CHINA, Beijing, Yanqing District, Yudu Mountain, 40.54399°N, 115.893984°E, alt. 860 m, 12 Sep 2018, coll. C.L. Hou, H Zhou and J.Q. Li, BJTC 646.

Discussion

In this study, three new species were identified by morphology and phylogeny. It is very interesting that the three new species *C. yanshanensis*, *C. subcandolleanus* and *C. incanus* formed a stronger supported clade and they clustered with *Candolleomyces badiophyllus* (Romagn.) D. Wächt. & A. Melze, *Candolleomyces candolleanus*,

Candolleomyces badhyzensis (Kalamees) D. Wächt. & A. Melzer, *Candolleomyces trinitatensis* (R.E.D. Baker & W.T. Dale) D. Wächt. & A. Melzer and *Candolleomyces cladii-marisci* (Sicoli, N.G. Passal., De Giuseppe, Palermo & Pellegrino) J.Q. Yan together in the phylogenetic tree. In addition, three new species were weakly sister to the known species *C. badiophyllus* in the phylogenetic tree.

Candolleomyces yanshanensis and *C. subcandolleanus* are different in macroscopic morphology of basidiomata. *Candolleomyces yanshanensis* is lighter in pileus colour and *C. yanshanensis* has larger spores ($5.8\text{--}8.2 \times 3.3\text{--}5.4$ vs. $5.5\text{--}6.7 \times 3.2\text{--}4.5$ μm) and longer cheilocystidia ($22\text{--}35 \times 8\text{--}11$ vs. $21\text{--}28 \times 8\text{--}12$ μm) than those of *C. subcandolleanus*. Moreover, *C. yanshanensis* spores often have a germ pore. *Candolleomyces subcandolleanus* is very easily confused with *C. candolleanus* in the field because of their similar macroscopic characteristics. In particular, two species in these sections possess the combined characteristics of small basidiomata. *C. candolleanus* is the type species of *Candolleomyces*, with early studies on this species being based on the number of pleats and other characteristics, but this also led to confusion in the identification of this species. *Candolleomyces subcandolleanus* can be distinguished from *C. candolleanus* by the smaller spores ($5.5\text{--}6.7 \times 3.2\text{--}4.5$ vs. $7\text{--}9 \times 4\text{--}5$ μm) (Kits van Waveren 1980; Breitenbach and Kränzlin 1995; Mifsud 2017).

Candolleomyces incanum, *C. badiophyllus*, *C. candolleanus* and *C. badhyzensis* are close to each other in the phylogenetic tree. However, the four species show significant differences in morphology. These species can be distinguished as follows: *C. incanum* has smaller and narrower spores ($6.0\text{--}7.0 \times 3.2\text{--}4.5$ μm), whereas *C. candolleanus*, *C. badhyzensis* and *C. badiophyllus* have larger spores (Spores of *C. candolleanus* were $7.0\text{--}9.0 \times 4.0\text{--}5.0$ μm , spores of *C. badhyzensis* were $10.2\text{--}11.5 \times 5.5\text{--}6.5$ μm , spores of *C. badiophyllus* were $10\text{--}14 \times 5\text{--}6$ μm). In addition, *C. incanum* has smaller cheilocystidia ($17\text{--}27 \times 7\text{--}11$ vs. $34\text{--}51 \times 10\text{--}15$ μm) than those of *C. badhyzensis* (Kalamees 1981; Kasik et al. 2004; Wächter and Melzer 2020).

Except for morphological differences, the three new species in this study can also be distinguished by sequence similarity. *Candolleomyces yanshanensis* (BJTC Z110) can be distinguished, based on nucleotide differences in ITS, LSU, *Tef1a* and *TUB2* loci from *C. subcandolleanus* (BJTC Z239) (sequence base similarity 93% in ITS, 100% in LSU, 99% in *Tef1a* and 98% in *TUB2*); *C. yanshanensis* (BJTC Z110) can be distinguished, based on nucleotide differences from *C. incanum* (BJTC Z777) (sequence base similarity 80% in ITS, 99% in LSU, 99% in *Tef1a* and 96% in *TUB2*); *C. subcandolleanus* (BJTC Z239) can be distinguished, based on nucleotide differences from *C. incanum* (BJTC Z777) (sequence base similarity 81% in ITS, 99% in LSU, 99% in *Tef1a* and 98% in *TUB2*). It can also be found that the ITS loci have a greater degree of differentiation for the species in *Candolleomyces*, Nevertheless, LSU and *Tef1a* were more conservative for the genus.

According to the research of Wächter and Melzer (2020), the species of *Candolleomyces* may be more abundant than previously thought and better delimitation of species boundaries is required. While the boundaries of some species are disputed, the number of new taxa is steadily increasing (Sicoli et al. 2019; Büttner et al. 2020; Bau and Yan 2021). However, the continued discovery of clear boundaries in new taxa like this study enhances our comprehension of species in this genus.

It is considered that the natural growth of *Candolleomyces* may be related to precipitation. However, the investigation and specimen collection in this study were carried out in the rainy season in July to August, with no collection in other periods. Therefore, more species of *Candolleomyces* might be expected in Yanshan Mountains.

Acknowledgements

This study was supported by the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment, China. (2019HJ2096001006) and National Natural Science Foundation of China (No. 31870629).

Reference

- Battistin E, Chiarello O, Vizzini A, Örstadius L, Larsson E (2014) Morphological characterization and phylogenetic placement of the very rare species *Psathyrella sulcatotuberculosa*. *Sydowia* 66(2): 171–181. [https://doi.org/10.12905/0380.sydowia66\(2\)2014-0171](https://doi.org/10.12905/0380.sydowia66(2)2014-0171)
- Bau T, Yan JQ (2021) Two new rare species of *Candolleomyces* with pale spores from China. *MycoKeys* 80: 149–161. <https://doi.org/10.3897/mycokeys.80.67166>
- Breitenbach J, Kränzlin F (1995) *Pilze der Schweiz* 4. Mykologia, Luzern.
- Büttner E, Karich A, Nghi DH, Lange M, Liers C, Kellner H, Hofrichter M, Ullrich R (2020) *Candolleomyces eurysporus*, a new *Psathyrellaceae* (*Agaricales*) species from the tropical Cúc Phương National Park, Vietnam. *Asian Journal of Mycology* 28: 79–92. <https://doi.org/10.21203/rs.3.rs-57408/v1>
- Fries E (1838) *Epicrisis Systematis Mycologici. seu synopsis Hymenomycetum*. Uppsala 4: 44–45.
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* 59(3): 307–321. <https://doi.org/10.1093/sysbio/syq010>
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42(2): 182–192. <https://doi.org/10.1093/sysbio/42.2.182>
- Hopple Jr JS, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: Divergent domains, outgroups, and monophyly. *Molecular Phylogenetics and Evolution* 13(1): 1–19. <https://doi.org/10.1006/mpev.1999.0634>
- Kalamees K (1981) Agaric fungi of Badhyz Nature Reserve. *Folia Cryptogamica Estonica* 15: 5–8.
- Kasik G, Dogan HH, Öztürk C, Aktas S (2004) New records in *Coprinaceae* and *Bolbitaceae* from Mut (Mersin) District. *Turkish Journal of Botany* 28: 449–455.
- Katoh K, Toh H (2010) Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics (Oxford, England)* 26(15): 1899–1900. <https://doi.org/10.1093/bioinformatics/btq224>

- Kits van Waveren E (1980) Checklist of synonyms, varieties and forms of *Psathyrella candolleana*. Transactions of the British Mycological Society 75(3): 429–437. [https://doi.org/10.1016/S0007-1536\(80\)80123-9](https://doi.org/10.1016/S0007-1536(80)80123-9)
- Kits van Waveren E (1985) The Dutch, French and British species of *Psathyrella*. Persoonia 2: 1–284.
- Larsson E, Örstadius L (2008) Fourteen coprophilous species of *Psathyrella* identified in the Nordic countries using morphology and nuclear rDNA sequence data. Mycological Research 112(10): 1165–1185. <https://doi.org/10.1016/j.mycres.2008.04.003>
- Matheny PB, Curtis JC, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Danielle G, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS (2006) Major clades of Agaricales: A multi-locus phylogenetic overview. Mycologia 98(6): 982–995. <https://doi.org/10.1080/15572536.2006.11832627>
- Mifsud S (2017) Contribution to the Mycobiota and Myxogastria of the Maltese islands. Part I (2014–2016). Micologia e Vegetazione Mediterranea 32(1): 3–58.
- Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémenton H, Miller OK (2002) One hundred and seventeen clades of euagarics. Molecular Phylogenetics and Evolution 23(3): 357–400. [https://doi.org/10.1016/S1055-7903\(02\)00027-1](https://doi.org/10.1016/S1055-7903(02)00027-1)
- Nagy LG, Walther G, Házi J, Vágvolgyi C, Papp T (2011) Understanding the evolutionary processes of fungal fruiting bodies: Correlated evolution and divergence times in the Psathyrellaceae. Systematic Biology 60(3): 303–317. <https://doi.org/10.1093/sysbio/syr005>
- Örstadius L, Kundsén H (2012) *Psathyrella* (Fr.) Quél. In: Knudsen H, Vesterholt J (Eds) Funga Nordica. Agaricoid, boletoid, cyphelloid and gasteroid genera. Nordsvamp, Copenhagen, 586–623.
- Örstadius L, Ryberg M, Larsson E (2015) Molecular phylogenetics and taxonomy in Psathyrellaceae (Agaricales) with focus on psathyrelloid species: Introduction of three new genera and 18 new species. Mycological Progress 14(5): 1–42. <https://doi.org/10.1007/s11557-015-1047-x>
- Posada D, Crandall KA (1998) Modeltest: Testing the model of DNA substitution. Bioinformatics (Oxford, England) 14(9): 817–818. <https://doi.org/10.1093/bioinformatics/14.9.817>
- Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. Journal of Molecular Evolution 43(3): 304–311. <https://doi.org/10.1007/BF02338839>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Sicoli G, Passalacqua NG, De Giuseppe AB, Palermo AM, Pellegrino G (2019) A new species of *Psathyrella* (Psathyrellaceae, Agaricales) from Italy. MycoKeys 52: 89–102. <https://doi.org/10.3897/mycokeys.52.31415>
- Smith AH (1972) The North America species of *Psathyrella*. The New York Botanical Garden 24: 1–633.
- Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics (Oxford, England) 22(21): 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>

- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57(5): 758–771. <https://doi.org/10.1080/10635150802429642>
- Swofford DL (2003) PAUP*: Phylogenetic analysis using parsimony (* and other methods). Version 4.0b10. Sunderland, England.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172(8): 4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- Wächter D, Melzer A (2020) Proposal for a subdivision of the family Psathyrellaceae based on a taxon-rich phylogenetic analysis with iterative multigene guide tree. *Mycological Progress* 19(11): 1151–1265. <https://doi.org/10.1007/s11557-020-01606-3>
- Wang Y, Bau T (2014) Well-known Chinese species of *Psathyrella* and their distribution. *Journal of Fungal Research* 12(3): 133–141. <https://doi.org/10.13341/j.jfr.2014.0053>
- Wang YT, Huang ZH, Wang J, Tong Z, Cui GF (2021) The population structure and dynamic characteristics of *Phellodendron amurense* in Yanshan Mountains. *Acta Ecologica Sinica* 47(7): 2826–2834. <https://doi.org/10.5846/stxb202003300743>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a Guide to Methods and Applications* 18: 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Yan JQ (2018) Taxonomy and molecular phylogeny of *Psathyrella* and related genera in China. Dissertation. Jilin Agricultural University.
- Yan JQ, Bau T (2017) New and newly recorded species of *Psathyrella* (Psathyrellaceae, Agaricales) from northeast China. *Phytotaxa* 321(1): 139–150. <https://doi.org/10.11646/phytotaxa.321.1.7>
- Yan JQ, Bau T (2018a) The northeast Chinese species of *Psathyrella* (Agaricales, Psathyrellaceae). *MycoKeys* 33: 85–102. <https://doi.org/10.3897/mycokeys.33.24704>
- Yan JQ, Bau T (2018b) *Psathyrella alpina* sp. nov. (Psathyrellaceae, Agaricales), a new species from China. *Phytotaxa* 349(1): 85–91. <https://doi.org/10.11646/phytotaxa.349.1.11>
- Yan JQ, Bau T (2021) A new and two newly recorded species in the /pygmaea clade of *Psathyrella* (Psathyrellaceae, Agaricomycetes) from China. *Junwu Xuebao* 40(3): 462–472. <https://doi.org/10.13346/j.mycosystema.200350>
- Yan JQ, Ge Y, Hu D, Zhou J, Huo GH (2019) *Psathyrella tintinnabula* sp. nov. (Psathyrellaceae, Agaricales), a new species from southwest China. *Phytotaxa* 400(2): 64–70. <https://doi.org/10.11646/phytotaxa.400.2.2>
- Zhou H, Hou CL (2019) Three new species of Diaporthe from China based on morphological characters and DNA sequence data analyses. *Phytotaxa* 422(2): 157–174. <https://doi.org/10.11646/phytotaxa.422.2.3>
- Zhou H, Wang QT, Tong X, Hou CL (2021) Phylogenetic analysis of *Engleromyces sinensis* and identification of cytochalasin D from culture. *Mycological Progress* 20(10): 1343–1352. <https://doi.org/10.1007/s11557-021-01739-z>